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Challenges in optimizing 3D scaffold for dentin-pulp complex regeneration

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Abstract: Regenerating dentin-pulp complex (DPC) using tissue engineering offers a novel and promising therapeutic alternative for restoring teeth. A crucial component of such a therapy is the designing and fabrication of an appropriate 3D Scaffold. In this review, we set out to highlight some of the general challenges associated with optimizing the most suitable scaffold for DPC regeneration to develop "bio-mimetic" approaches that influence stem cell proliferation, differentiation, and angiogenesis. It is essential to comprehend the biology and physical features of the dentin-pulp complex with updated bionanotechnology to overcome the limitations of biomaterials to address the challenges in manufacturing the optimal scaffold. To date, current scaffolding models fail to regenerate a whole tooth. The success of regenerative dentistry relies on stem cells and scaffolds may shape the future of dental treatment.

Keywords: Tissue engineering ;3D scaffold; Pulp dentin complex; Regenerative dentistry; Stem cell therapy

1 Introduction

Tissue engineering is an interdisciplinary topic that combines cell biology understanding, biotechnology, biomaterials, and appropriate biochemical components to manufacture replica organs and tissues or to restore damaged tissues (Langer 2007; O'Brien 2011). It necessitates seeding cells onto such a scaffold, which is then grown in vitro before being transplanted into the body when matured (Rabkin and Schoen 2002) following that, normal tissue regeneration processes occur, blood vessels enter the structure, and angiogenesis is completed (Noohi et al. 2022) and the scaffold finally dissolves, degradation results in newly regenerated tissue in the site (Li et al. 2019). That's why it is important to first understand and replicate tissues' dynamic processes to repair injured tissues and organs (Jain and Bansal 2015; Gritsch et al. 2019). It wasn't until the late 1980s that the term "tissue engineering" entered the terminology. Synthetic biodegradable materials are often known as scaffolds. The scaffold replicates the in vivo environment by providing a three-dimensional area for cell growth (Almutairi et al. 2019). These synthetic matrices may be built to any shape and include growth agents to differentiate cells into the right tissue types as shown in the steps of Figure 1 (Huang 2009).

Over the past two decades, tissue engineering has advanced in biodegradable scaffolds, integration of cells and biopolymers to create tissue engineering constructs, bioreactors that stimulate cultured tissues with developmentally relevant signals, and characterization and isolation of adult and embryonic stem cells (Qu et al. 2015; Diana et al. 2020). Clinical advancements have occurred with skin and cartilage, which are simple to replace. Despite these successes, many obstacles remain (Liu et al. 2007).

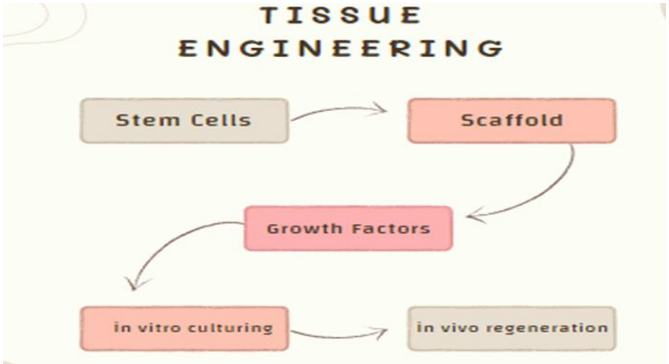

Fig 1. Tissue engineering procedure.

2 Tissue Engineering Scaffold features

The scaffold supports cell attachment, proliferation, differentiation, and extracellular matrix (ECM) formation. ECM plays a role in cell-to-cell interactions and tissue architecture. Scaffolds shuttle cells, growth factors, as well as biomolecular signals (Agrawal and Ray 2001; Chan and Leong 2008; Zhang et al., 2019).

Scaffolds, a crucial part of the tissue engineering concept, address tissue creation by providing cells with a suitable environment in which to adhere, proliferate, differentiate, and produce their extracellular matrix (O'Brien 2011).

Generally, the optimum scaffold should have distinctive features. First, cell migration and division are facilitated by a dense network of linked holes attached throughout the scaffold's interior. Porous structures with channels allow for the efficient transport of oxygen and the amount of nutrients to cells at different depths within the scaffold, as well as the removal of metabolic waste. Biocompatibility requires just a tight fit for cells to attach and grow, designed to the required tissue. An adequate biodegradation rate and the right amount of mechanical strength and physical characteristics are other substantial properties. Utilizing such a scaffold would greatly benefit tissue engineering and regeneration. Biomaterials utilized in tissue engineering scaffold manufacturing may be broadly classified as synthetic or natural sources, or semisynthetic materials (Griffith and Naughton 2002; Liu et al. 2007; O'Brien, 2011; Elline et al. 2022).

To yet, tissue engineering products have only been successful in thin structures (skin) and tissues without a blood supply (cartilage) (Cao et al. 2006). However, several strategies are being studied for the development of scaffolds to compensate for deficiencies in other tissues.

The primary prerequisites for biomaterials used as scaffolds include biocompatibility as well as adequate surface characteristics to promote cell adhesion, proliferation, and differentiation (Dhandayuthapani et al. 2011; Leite et al. 2021).

For scaffold production in diverse tissue engineering applications, synthetic biomaterials (bio-ceramics & biopolymers) are the most commonly used (Liu et al. 2007). Natural or manufactured synthetic biomaterials also serve as scaffolding. Porosity, mechanical properties stability feasible application for clinical treatment are all important features of an optimal scaffold for successful tissue regeneration (Palma et al. 2017).

In literature, pulp tissue regeneration failed in the 1960s and 1970s. Tissue-engineering approaches in the late 1990s re-evaluated pulp tissue regeneration (Huang 2009). In immunocompromised mice, Gronthos et al. showed that pulp cells may form dentin (Gronthos et al. 2000) this discovery illuminated therapeutic pulp dentin regeneration.

In the last decade, the pulp-dentin complex and its regeneration have grown in relevance and complexity. To date, several biomaterial scaffolds from various cell sources have been suggested to regenerate natural extracellular matrix (ECM) analogs. Adequate mechanical qualities and the capacity to promote cell adhesion and proliferation are prerequisites for a successful dental scaffold design (Zhang et al. 2013; Grawish et al. 2020; Iranmanesh et al. 2022).

The present study aims to draw attention to some of the challenges that arise when we want to figure out the optimal scaffold to regenerate the dentin pulp complex.

3 Scaffolds to regenerate dentin-pulp complex

Prosthetics and implants with prosthetic crowns are the main dental treatments for missing teeth. The prostheses don't remodel like normal teeth. Nowadays the demand uses of postnatal dental stem cells on a bioengineered three-dimensional framework to regenerate tooth organogenesis is increasing (Zhang et al. 2013).
In teeth, pulp and dentin are considered histologically as two different tissues functionally as one entity (Linde and Goldberg 1993).

Dentin, nerves, as well as blood vessels, are all produced by odontoblast cells throughout development. While dentin and pulp feature separate in their structures and compositions once formed, they remain responsive to signals as just a single entity. Dentin exposure caused by attrition, trauma, or caries causes severe pulpal responses that lower dentin permeability and encourage dentin production, the alteration of fibroblasts, nerves, blood vessels, odontoblasts, leukocytes, and immune systems triggers these responses (Goldberg and Smith 2004; Tsou et al. 2017).

Recent findings of the impact of nerves upon the pulp's blood vessels conversely have given us a more complete understanding of how they interact depending on the dentin's response to the stimulus. Too frequently in works of literature, the various components of the pulp-dentin complex are investigated separately. However, the separate components are highly interacting, with each moderating the performance of the others. While delving into the intricacies of the pulp-dentin complex may pose challenges in the realm of research, it nonetheless presents an exceptional and distinctive backdrop for scholarly inquiry.

Scientists use multidisciplinary methodologies to address the challenges related to the pulp-dentin complex. Twenty years ago, the finding of stem cells in dental pulp opened the possibility for the regeneration of dentin pulp complex (Gronthos et al. 2000).

Some consider the predicted regeneration of the dentine-pulp complex to be the "golden standard" of regenerative research, however, this has yet to be accomplished. One of the challenging targets in regenerating the complex is gaining the unique tubular design that is only found in the calcified natural dentin (Tsou et al. 2017).

Most of the dentin pulp complex regeneration literature lacks definitive data on the optimal biomaterial scaffold, leaving the field open to trial-and-error approaches. The proper biomaterials are essential for regenerating teeth to function like the original. The literature evaluation also lacks pulp tissue material characteristics data (Ozcan et al. 2016).

4 Difficulties Associated with Utilization Scaffolds in Dental Regeneration

Fig 2. The main challenges in utilizing an optimal scaffold.

The most challenging aspect of finding the optimal scaffold is selecting the suitable biomaterial that will be employed. Recent works of literature contain material suggestions and primary assessments (Farag 2022).

Blood clots have been used as scaffolds for some studies (Vail et al. 1999). However, numerous scaffolds—natural polymers (collagen, fibrin, chitosan, alginate) or synthetic polymers have been used in investigations (PGA, PLGA, PLA). Synthetic biopolymers are more versatile and predictable than natural materials. Several studies also focused on FDA-approved synthetic biopolymers. Polylactic acid (PLA) and poly-glycolic acid (PGA) are the most often studied biodegradable polymers (Leite et al. 2021; Elline et al. 2022).

Degradation and by-products are one of the limitations of synthetic polymers (PLA, PGA, PLGA, PLLA) that disintegrate rapidly after implantation. Polymers lose molecular weight in liquids. Mass loss can't occur until molecular chains are small enough to diffuse out of the polymer matrix. Acidic by-products release gradient-wise with mass loss. The observed transitory abnormalities may be caused by a massive release of acidified breakdown and resorption by-products, which may cause inflammatory reactions in vivo if the adjacent tissue has inadequate vascularization or metabolic activity (Hutmacher 2000; Anderson 2001; Rodrigues et al. 2016). Tissue-engineered products may be impeded by inflammation and foreign body reactions.

Incorporating ceramics like hydroxyapatite (HA) particles into a polymer matrix creates a composite scaffold with decreased local inflammatory reactions was one of the overcoming. HA's basic resorption affects buffer polymers' acidic degradation by-products, shielding cells from a hazardous environment (Kempen et al. 2006). Coating by such particles should not impair the polymer's mechanical qualities or biocompatibility.

The model, cell exclusion approaches, stem cell potentials, micro-environment, teeth, in situ microcirculation, surgical process, and biomaterials are all factors that affect study outcomes. inflammation may also hinder neo-angiogenesis and mineralized tissue development, which contribute to regeneration defects (Peters et al. 2021).

Acellular alternatives have been created to avoid cell transplantation's drawbacks. Acellular approaches emphasize cell recruitment with full pulp regeneration relying on periapical cells. Trope achieved the most clinical improvement utilizing this strategy. After cleaning the canal, instrumenting into the peri-apical region creates a blood clot. The blood clot forms new tissue and stores natural biomolecules that attract new cells (Trope 2010).

In creating an artificial organ, biomaterial surfaces have become essential (Leite et al. 2021). Biodegradable scaffolds bring cells together for microenvironments, scaffolds are optimized for physical, mechanical, biochemical, and biological qualities. Voltage may be used to create surfaces with dynamic interfacial properties, including wettability. Surface-confined, single-layered molecules translate between hydrophilic and moderately hydrophobic states to modify wetting behavior (Langer 2007; Hollister and Lin 2007).

A decellularized matrix with specified trophic element distribution is intriguing. Extracellular matrix-based scaffolds have modulated the host response, attracted progenitor cells, and stimulated constructive remodeling in recent investigations. A decellularized matrix attracts stem cells and progenitors. Regeneration in those investigations parallels natural cell recruitment from neighboring tissues to the new ECM network (Peters et al. 2021).

The ubiquitous need for creating blood perfusion via engineered tissues of clinically meaningful met by tissue vascularization which can be utilized to initiate or restore blood flow is another challenge (Langer 2007) sustained release of angiogenic factors through scaffolds, seeded endothelial cells straight into the scaffold, and designing vasculature directly into tissue via microfabrication are promising approaches to this challenge (Noohi et al. 2022).

Stem cell sources are a significant challenge in the field of dental tissue engineering. Dental pulp stem cells found in permanent and deciduous teeth may be exploited for tissue engineering. The first effective attempt to build complex tooth designs using single-cell solutions separated from swine third molar tooth bud showed dental pulp stem cells throughout this tissue (Gronthos et al. 2000) Since then, similar methods using self-dental pulp stem cells as bioengineered organs for regenerative investigations are an option.

Sharpe and Young (2015) demonstrated that both non-dental and dental adult stem cells have the capability to generate mouse teeth, emphasizing the potential utility of non-dental stem cells in dental research.

Mesenchymal stem cells from the root apical papilla were shown to rebuild tooth strength and appearance (Cordeiro et al. 2008). However, cell differentiation and stem cell sources require further research.

Tissue engineering of the dentin-pulp complex presents several challenges when using 3D scaffolds. Scaffolds must do more than support cells mechanically to resemble the extracellular matrix. It must also be bioactive and dynamic, regulating cellular activity and intercellular communication (Gong et al. 2016). Electrospinning, gas foaming, melt

molding, freeze-drying, solvent casting, particle leaching, and phase separation are traditional scaffolding processes that cannot be accurately controlled (Peters et al. 2021).

Processes such as choosing the biomaterial with appropriate physicochemical properties, producing the designed scaffold, and then monitoring the response in the tissue have separate difficulties and risks (Figure 2).

5 3D printed scaffold challenges dental regeneration

Another crucial challenge in optimizing the scaffold is the manufacturing procedure. Recent years have seen the introduction of additive manufacturing methods like 3D printing into the field of tissue engineering (Noohi et al. 2022). The idea of 3D bioprinting stems from Charles Hull's development of stereolithography in 1986. Customized cell-laden scaffolds are the result of 3D bioprinting. Cells may be placed with pinpoint accuracy using 3D bioprinting (Figure 3). It may be employed for high throughput manufacture and provides fine control over scaffolds' exterior and interior morphology. Because of its porous interior, the 3D-printed scaffold can allow nutrients and oxygen to reach the cells inside it, promoting healthy cellular metabolism (Peters et al. 2021).

Fig 3. The 3D dental bioprinting procedure

Endodontic regeneration bioprinting options include inkjet bioprinting, laser-assisted bioprinting (LAB), as well as extrusion bioprinting (Figure 4) which are the main types of 3D bioprinting (Iranmanesh et al. 2022). Relatively common 2D desktop inkjet printers have helped popularize the printing process known as "inkjet printing." Droplets of biomaterials are ejected from the nozzle of an inkjet printer through either heat energy or a piezoelectric actuator. It's easy to use, produces high-quality results, and won't break the bank: thermal inkjet technology. Yet, bio-tendency inks to clog nozzles is a significant drawback of this technique. As a result of gelation, droplets are not uniform in size, which might pose problems during the printing process. Another difficulty arises from the fact that the heat and shear stress caused by making bio-ink drops might affect cell viability (Matai et al. 2020).

In LAB, laser pulses at a donor slide propel cell-loaded hydrogel droplets toward a collection slide (Vijayavenkataraman et al. 2017). LAB makes heterogeneous tissue structures with high precision (10-100 m), a broad variety of sizes, and high cell density. Automation, reproducibility, and high productivity make LAB a potential 3D tissue creation process. Biomaterials must cross-link quickly, so should be chosen carefully. Laser wavelength must preserve cell and biomaterial resolution in 3D printed constructs (Matai et al. 2020). However, manufacturing time and high-pressure homogenization of cells in solution are major concerns (Peters et al. 2021).

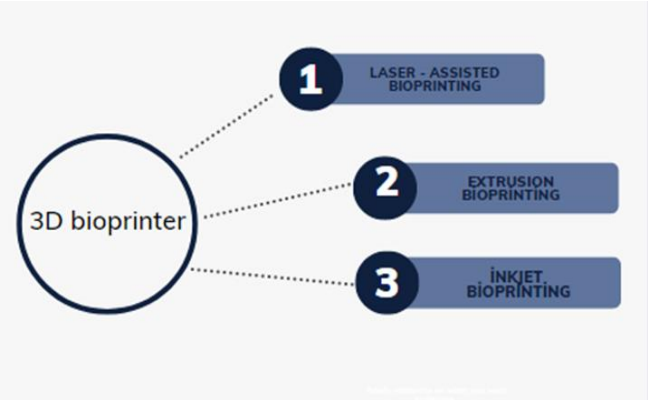

Fig 4. The main type of 3D bioprinter

Extrusion bioprinters propel biopolymers or cell-laden hydrogels via a nozzle using air pressure or mechanical devices like pistons or screws. Extrusion bioprinters with several printer heads may deposit multiple bio-inks without cross-contamination. They enhance printed structure porosity, shape, and cell dispersion. Extrusion bioprinting for tissue engineering scaffolds is becoming more popular due to its versatility (Matai et al. 2020; Peters et al. 2021; Iranmanesh et al. 2022)

Despite its rising popularity due to its benefits and uses in other medical sectors, only a few research publications have been published on 3D bioprinting for endodontic regeneration. 3D bioprinting for dental regeneration is yet to be optimized. Use which bioprinter technology? Which bio-ink is better for endodontics? Which method—fabricating dental pulp, tissue structures, enamel, cement, and ligament—would provide the greatest results? These printers employ cell-laden hydrogels, extracellular matrix, or cell aggregation bio-inks. These materials don't imitate dentine and pulp's intricate extracellular matrix. Bio-inks that regenerate tissues, especially odontogenic ones, may help regenerative dentistry grow.

Bio-ink odontogenic and cytocompatibility have been studied lately. The differentiation and proliferation of DPSCs in a regular Alg-Gel hydrogel (Alginate-gelatine) was compared to the scaffold to a 3D bioprinted scaffold extract. 3D-printed Alg-Gel scaffolds develop and adhere faster than conventional ones. Seeded DPSCs show increased proliferation and osteogenic/odontoblastic differentiation potential because 3D-printed Alg-Gel scaffold extracts contain more phosphorus and calcium (Yu et al. 2019).

Cell-loaded collagen-based bio-inks with the necessary biological properties and structure may be bioprinted in the root canal. A portable drop-on-demand bioprinter was utilized to test ex vivo human teeth. Vasculogenesis, the production of new blood vessels, was shown to be qualitatively and quantitatively identical to collagen, fibrin, and non-bio-printable hydrogel controls (Duarte Campos et al. 2020).

In studies for complete tooth regeneration, tooth-like tissues and structures with different properties have been created. Research data showed that smaller sizes of dental tissue could be produced instead of the entire crown of the tooth. Dental germ culture is difficult (Li et al. 2019) and scaffold-based methods are unsuited for tooth-like tissue regeneration because they can't pre-define the positioning of many cell types (Peters et al. 2021).

Dentine-pulp complex reconstruction using 3D bioprinting is new. Before they can be used securely and cost-effectively, the approaches require additional study and development. Most evidence comes from in vivo and ex vivo models, which do not account for diagnostic and therapeutic parameters, microorganisms and their by-products, dentin's inherent structure, or the impact of irrigant solutions on the remaining dentin. No controlled clinical studies of these regenerative dental treatments have been done (Peters et al. 2021; Iranmanesh et al. 2022).

6 Conclusion

It is essential to comprehend the biology and physical features of the dentin-pulp complex with updated bionanotechnology to overcome the limitations of biomaterials in order to address the challenges in manufacturing the optimal scaffold. Utilizing components that contribute to normal tooth function and structure must comprehensively address the difficulties of generating a dentin-pulp complex that resembles the natural tissues to reach the whole bioengineered tooth.

Since stem cells are employed in dentin pulp complex regeneration research, procedures should be given more attention. Bacteria may influence regeneration procedure outcomes.

Some stem cells are more susceptible to apoptosis and immune-mediated cell death. Thus, it is unclear whether these cell types may be exploited similarly. Stem cell characteristics and interactions require further study. Stem cells interact differently with the immune system. This knowledge is crucial for regenerative medicine. Stem cell treatment may be harmful if stem cells generate proinflammatory cytokines. The current scaffolding models for pulp dentin complex regeneration fail to account for the important variations between pulp and dentin and are thus unable to regenerate a whole tooth. However, in studies for regenerative endodontic treatment, the most effective results are obtained with stem cell research. There is a need for further research in the field to get complete regeneration, and address the challenges, overcoming the limitations in previous studies. Finding the optimal scaffold may alter the future of dental treatment methods. The bioengineered future is bright, and what we discover about stem cells and scaffolds today will shape it.

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